

1/29/98

'129 Screening "α-STNFR" Hybridomas Against native STNFR

The previous screening of the hybridomas was against denatured STNFR (pg. 150 Book #3). In that assay, all the hybridoma pupts. exhibited a positive signal. Ultimately, however, we want an antibody which recognizes the native protein. Therefore, we will now screen the hybridomas against captured, native STNFR from 9B supt.

Procedure

- 1) Coat 96-well plate w/ 2 μg/ml goat α hSTNFR Ab in 0.1M NaH₂SO₄/well. - Block in 5% BSA/PBS
- 2) Add 9B (W37+ pmHneo/hSTNFR) pupt - 50 μl/well
- 3) Add 50 μl of hybridoma pupt
- 4) Detect hybridoma Ab with goat α mouse - phosphatase Ab at 2 μg/ml.
- 5) Add 50 μl of pNPP substrate. Stop reaction w/ 50 μl 5% EDTA.

(+) control = immune serum from mouse immunized w/ denatured STNFR to make hybridomas.

(-) control = SP2/0 pupt. (Raw Abs = 0.250)

Plot #1

Plate #3

	1	2	3	4	5	6	7	8	9	10	11	12
1	0.040	0.755	0.174	0.237	0.063	0.195	0.147	0.140	0.145	0.301	0.244	0.190
2	0.127	0.190	0.764	0.157	0.158	0.143	0.113	0.153	0.205	0.225	0.245	0.266
3	0.192	0.102	0.704	0.220	0.119	0.102	0.118	0.072	0.075	0.283	0.288	0.240
4	0.248	0.178	0.109	0.128	0.109	0.152	0.211	0.178	0.194	0.270	0.247	0.188
5	0.146	0.115	0.215	0.078	0.087	0.151	0.064	0.087	0.161	0.046	0.056	0.277
6	0.240	0.151	0.189	0.157	0.133	0.167	0.082	0.146	0.293	0.226	0.233	0.251
7	0.170	0.058	0.038	0.065	0.083	0.090	0.077	0.084	0.123	0.114	0.101	0.430+
8	0.243	0.210	0.212	0.116	0.157	0.021	0.058	0.088	0.044	0.018	0.010	0.000+

Ultra plate

	1	2	3	4	5	6	7	8	9	10	11	12
1012 A	0.375	0.4075412	0.079	0.067	0.090	0.093	0.136	0.114	0.148	0.116	0.107	0.107
1812 B	0.537	0.4985412	0.156	0.109	0.153	0.173	0.333	0.090	0.190	0.069	0.270	0.179
2612 C	0.368	0.389+	0.211	0.177	0.196	0.252	0.169	0.084	0.078	0.118	0.179	0.131
2812 D	0.380	0.230+	0.153	0.206	0.181	0.160	0.118	0.144	0.179	0.012	0.131	0.131
3412 E	0.437	0.270	0.049	0.184	0.170	0.179	0.175	0.084	0.084	0.084	0.188	0.188
3812 F	0.274	0.259	0.118	0.170	0.165	0.134	0.155	0.043	0.094	0.147	0.145	0.145
4412 G	0.432	0.354	0.084	0.073	0.087	0.141	0.101	0.140	0.085	0.142	0.405+	0.405+
4412 H	0.318	0.271	0.181	0.081	0.133	0.212	0.123	0.124	0.144	0.183	0.000+	0.000+

Take all hybridomas above 0.3 Abs. units as positives to screen in TRF neutralization assay. Rescreen remaining. Note: The only one that is positive on the "Ultra plate" is 1H12 - the report above does not have the background subtracted out. See the plate outline on the reverse for the list of the positives.